

METHOD OF DESIGNING TUBULIN POLYMERIZATION STABILIZERS

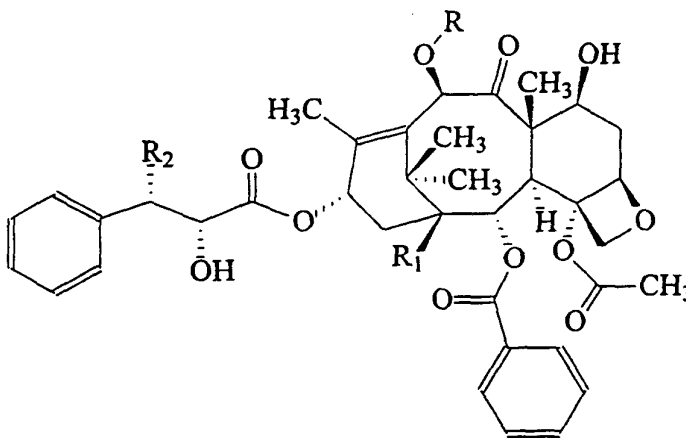
TECHNICAL FIELD OF INVENTION

This invention relates a method of designing paclitaxel alternative compounds.

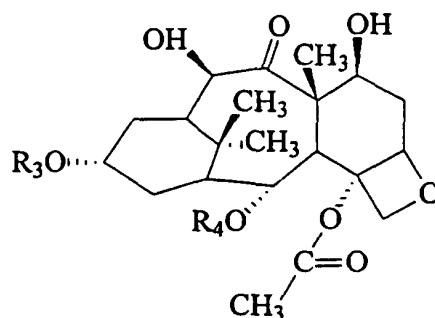
BACKGROUND

Paclitaxel is a complex diterpenoid sold commercially as TAXOL® (Bristol-Myers-Squibb). Paclitaxel and many of its derivative have been reported to possess potent antileukemia activity as well as significant anticancer activity against carcinomas of the ovaries, breast, lung, bladder, esophagus, head, and neck. (Rowinsky, E.K. and Donehower, R.C. 1991. "The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics," *Pharmacol Ther* 52:35-84; and Rowinsky, E. K. 1994. "Update on the antitumor activity of paclitaxel in clinical trials," *Ann Pharmacother* 28(5 Suppl): S18-22).

Paclitaxel and its derivatives are represented by the following chemical formula. For paclitaxel, R = acetyl; R₁ = OH; and R₂ = NHCOC₆H₅. For the derivative docetaxel, R = OH; R₁ = OH; R₂ = NHCOOC(CH₃)₃.



The structure for the taxane skeleton is represented as follows:



The mechanism of action for paclitaxel has been previously determined. (Derry, et al. 1997. "Taxol differentially modulates the dynamics of microtubules assembled from unfractionated and purified beta-tubulin isotypes," *Biochemistry* 36:3554-3562). Its anti-tumor activity is due to its ability to bind to beta-tubulin in assembled microtubules and stabilize them (Manfredi, J.J. and Horwitz, S.B. 1984. "Taxol: an antimetabolic agent with a new mechanism of action," *Pharmacol Ther* 25:83-125; and Horwitz, S.B. 1992. "Mechanism of action of taxol," *Trends Pharmacol Sci* 13:134-136). *In vivo*, paclitaxel affects spindle function during mitosis, resulting in cell cycle arrest in G2/M phase. *In vitro*, paclitaxel promotes microtubule assembly and prevents their disassembly under conditions which would otherwise cause depolymerization (Schiff, et al. 1979. "Promotion of microtubule assembly in vitro by taxol" *Nature* 277:665-667; and Parnass, J. and Horwitz, S.B. 1981 "Taxol binds to polymerized tubulin *in vitro*," *J Cell Biol* 91:479-487).

The x-ray structure of paclitaxel bound to its receptor has been previously reported. (Nogales, et al. 1998. "Structure of the alpha beta tubulin dimer by electron crystallography," *Nature* 391:199-203; erratum published in *Nature* 393:191). The alpha-beta heterodimer is the structural subunit of microtubules, which are cytoskeletal elements essential for intracellular transport and cell division in eukaryotes. The structures of alpha- and beta-tubulin are identical with each monomer being formed by a core of two beta-sheets surrounded by alpha-helices. The monomer structure is divided into three functional domains: the amino-terminal domain containing the nucleotide-binding region, an intermediate domain containing the

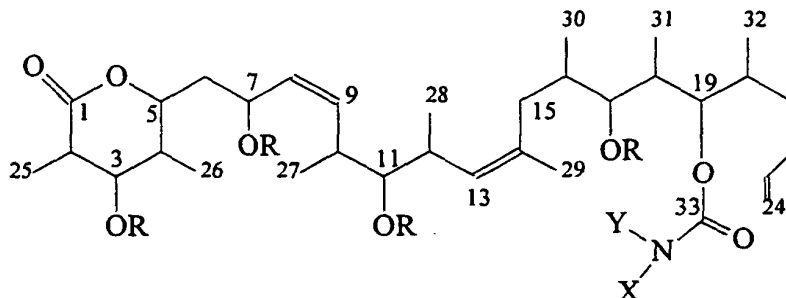
paclitaxel-binding site, and the carboxy-terminal domain, which is reported to constitute the binding surface for motor proteins.

Despite the anticancer and antileukemia activity of paclitaxel, there are distinct disadvantages of using paclitaxel as a therapeutic agent. For example, the synthesis and/or production of paclitaxel from natural sources is very complex and costly. Paclitaxel is highly toxic, often being toxic at therapeutic levels. Paclitaxel's low solubility causes complications in preparation and administration of therapeutic dosages.

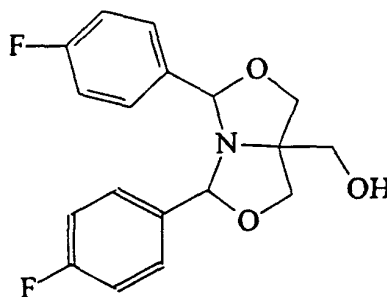
Numerous patents have issued which disclose paclitaxel derivatives in which substitutions have been made on the taxane skeleton. For example, U.S. Patent No. 4,814,470 issued March 21, 1989, discloses paclitaxel derivatives which reportedly have anticancer activity greater than paclitaxel itself. Referring to the chemical formula given above, the chemical formulas for these derivatives consist of R = H or acetyl; R₁ = OH or tert-butoxycarbonylamino; and R₂ = OH when R₁ = tert-butoxycarbonylamino or R₂ = tert-butoxycarbonylamino when R₁ = OH. U.S. Patent No. 4,206,221 issued June 3, 1980, discloses paclitaxel derivatives for the remission of leukemia in animals. Referring to the chemical formula given above, the chemical formulas for these derivatives consist of R = acetyl; R₁ = OH; and R₂ = NHCOCCH₃CHCH₃. Among other examples are U.S. Patent No. 5,635,531 disclosing 3'-aminocarbonyloxy paclitaxels, U.S. Patent No. 5,912,264 disclosing 6-halo- or nitrate-substituted paclitaxels, U.S. Patent No. 6,017,935 disclosing 7-sulfur substituted paclitaxels, and U.S. Patent No. 5,977,386 disclosing 6-thio-substituted paclitaxels. By making substitutions on the taxane skeleton, derivatives demonstrating anticancer activity have been found.

Compounds have also been disclosed which are not paclitaxel derivatives but exhibit "paclitaxel activity", i.e., the inhibition of depolymerization of microtubules. A marine natural product (+)-discodermolide has been reported to possess tubulin-polymerizing and antitumor properties. (Hung, et al. 1996. "(+)-Discodermolide binds to microtubules in stoichiometric ratio to tubulin dimers, blocks taxol binding and

results in mitotic arrest," *Chem Biol* 3:287-293). The basic structure of discodermolide is as follows:



5 A synthetic anticancer agent known as GS-164 having the following chemical structure has been reported to stimulate microtubule polymerization.



Comparative conformational analysis reportedly indicated that the structure of GS-164 mimics the minimum essential sites of TAXOL® required to exhibit TAXOL®-like properties. (Shintani, et al. 1997. "GS-164, a small synthetic compound, stimulates tubulin polymerization by a similar mechanism to that of Taxol," *Cancer Chemother Pharmacol* 40:513-520.)

Disadvantages have also been associated with some paclitaxel derivatives. For example, many paclitaxel derivatives reported to date have not had the steric conformational properties of natural paclitaxel, nor has there been the ability to change the right side of the molecule by combinatorial chemistry with carbohydrates, calcium chelators, or oxygenated small molecules.

A method has been found by which compounds exhibiting paclitaxel-like activity and having distinct advantages over paclitaxel and known derivatives can be synthesized.

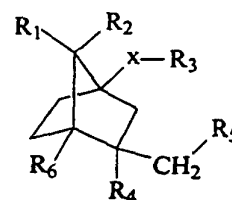
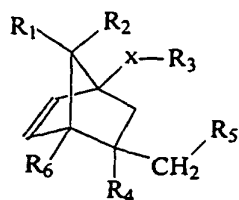
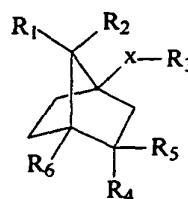
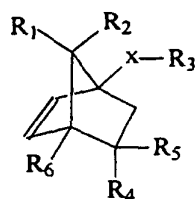
SUMMARY OF THE INVENTION

In one aspect, the present invention is a method for designing anti-tumor compositions, comprising: (a) using molecular modeling software on a computer to create a plot of an active conformation of a known anti-tumor composition, the active conformation representative of a three-dimensional conformation of the known anti-tumor composition interacting with a target biological site, the plot providing a first digital representation of the active conformation, the first digital representation depicting a plurality of binding sites of the known anti-tumor composition; (b) using the software to eliminate portions of the first digital representation while preserving the depiction of the binding sites; and (c) using the software to build a second digital representation of a created composition, the created composition having a three-dimensional conformation and binding sites similar to the known anti-tumor composition. Preferably, the known anti-tumor composition has a structure including a central skeleton which is depicted in the plot, and the software is utilized to eliminate the central skeleton from the depiction and to substitute therefore a second central skeleton having desired characteristics. More preferably, the known anti-tumor composition has a structure including a central skeleton and one or more original side chains which are depicted in the plot, and the software is utilized to eliminate one or more original side chains from the depiction and optionally to substitute a created side chain for one or more of the original side chains. The method can further comprise using the software to eliminate the central skeleton from the depiction and to substitute therefore a second central skeleton having desired characteristics. Preferably, a calculation is performed to determine a binding energy for the created composition, and the created composition is further modified to improve putative binding characteristics, wherein an improved binding characteristic is characterized by a higher binding energy. An exemplary known anti-tumor composition is paclitaxel.

In another aspect, the invention is a method for designing a paclitaxel alternative composition, which alternative composition has a central skeleton structure composed of single or multiple ring groups which hold multiple functional groups in a fairly rigid alignment, the central skeleton structure having first, second, and third side

chains; wherein the first side chain is connected to the central skeleton with a carbonyl group at a distance of about 1.5 to 5.5 Angstroms from the central skeleton; herein the second side chain places an sp^3 oxygen atom at a distance of about 4.5 to 7.5 Angstroms from the skeleton and about 9 to 11 Angstroms from the carbonyl oxygen of the first side chain; wherein the third side chain is placed in an energetically accessible conformation that places an aromatic ring in a location that is simultaneously about 4 to 6 Angstroms from a substitute for hexene and about 8 to 10 Angstroms from the oxygen in the second side chain, the third side chain selected to mimic the steric and binding properties of the C2 ester in paclitaxel; wherein the method comprises using molecular modeling software on a computer to design the alternative composition. The alternative composition can further comprise one or more bulking groups and wherein the bulking groups increase the size of the composition to mimic the overall size and shape of the paclitaxel molecule. Preferably, the first side chain is selected and positioned to mimic the isoserine group in taxane. Preferably, the sp^3 oxygen is positioned in space to simulate the position of the oxetane ring of paclitaxel. Preferably, the method further comprises synthesizing said alternative composition.

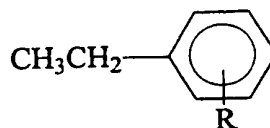
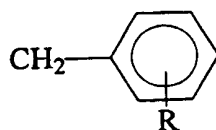
In another aspect, the invention is a paclitaxel compound having a chemical structure selected from one of the following norbornyls



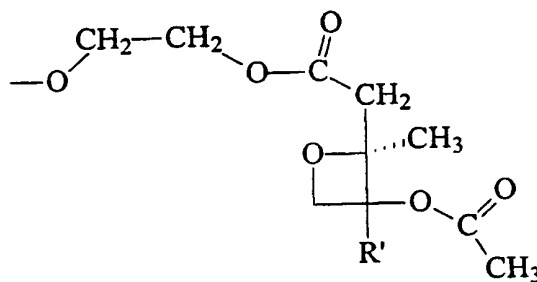
wherein R_1 or R_2 or both R_1 and R_2 are hydrogen, methyl, acetyl, ethyl, short aliphatic chain ($C_1 - C_4$), or substituted aliphatic chain ($C_1 - C_6$) where substitution includes in one or two of the R_1 organic functional groups such as an amide; ketone; hydroxy; phenyl; carboxylic acid; an amino acid, for example, asparagine, glutamine, aspartic acid, glutamic acid, threonine, serine or tyrosine. Preferable chemical structures are obtained with the following:

$R_1 = H$ or CH_3 ;

$R_2 = CH_3$; CH_2OCOCH_3 ; or



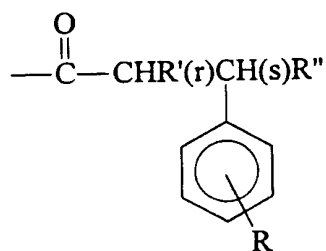
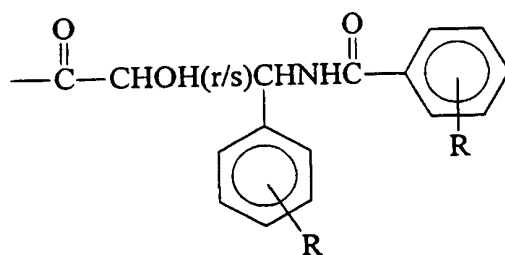
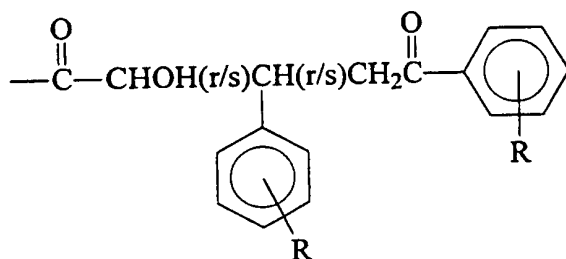
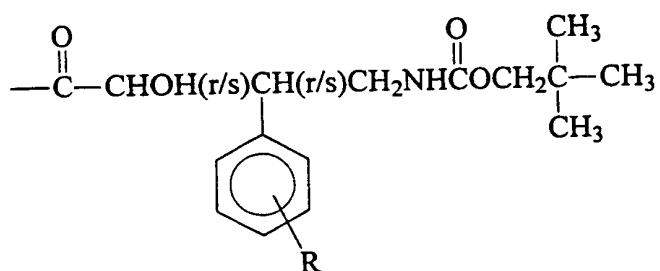
wherein R is H or singly, doubly, or triply substituted or fused; or



wherein R' is H or CH_3 ;

wherein $X = O$; CH_2 ; NH ; S ; $S-CH_2$; $O-CH_2$ or none;

wherein R_3 is one of the following:



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wherein R' = OH when R'' = NHBOC; R' = H when R'' = NHBOC; R' = OH when R'' = H; R' = H when R'' = H

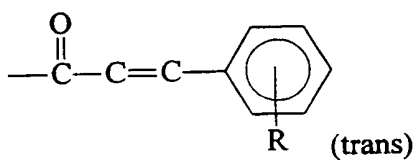
(These substituents are still active in paclitaxel per Guenard, et al. 1993. "Structure-activity relationships of taxol and taxotere analogues," *J Natl Cancer Inst Monogr* 15:79-82.);

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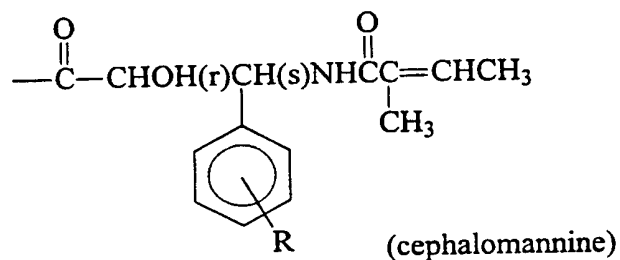


wherein R' is as given above and R'' can also be substituted aryl (single or double) or fused aromatic ring as in tryptophan or imidazol ring, or substituted tryptophan; preferably, the aromatic ring can be substituted with carboxylic acid derivatives;

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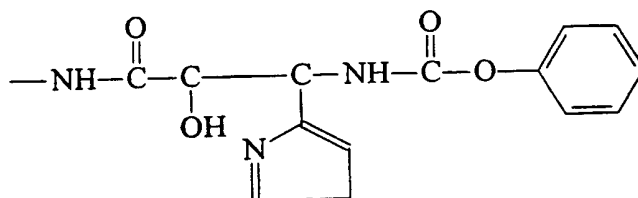


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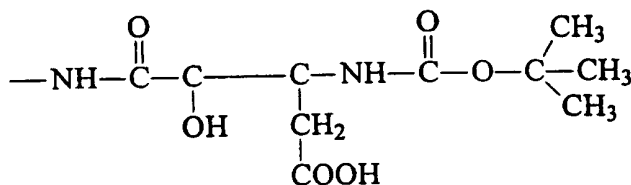
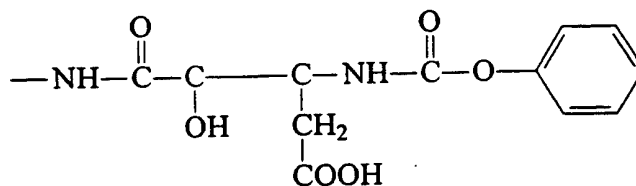


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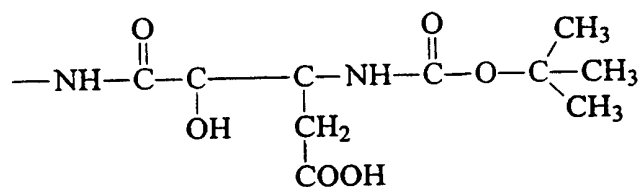
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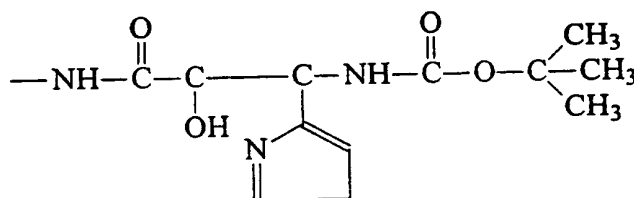
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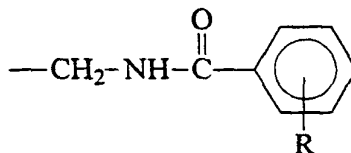
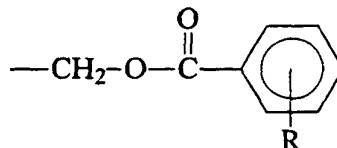
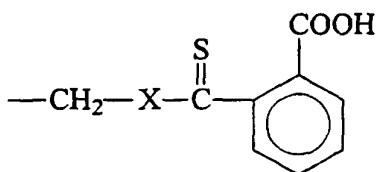


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For the aryl groups in R₃, R can be H or singly, doubly, or triply substituted OH or preferably with electron withdrawing substituents such as fluoro (F⁻) or trifluoromethyl (CF₃⁻). R₃ can also be any group derived from the 13 position in taxane's skeleton that exhibits activity toward inhibiting the depolymerization of microtubules and/or anticancer activity;

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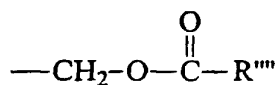
wherein R₄ is one of the following:



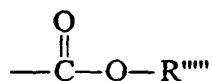
when the aromatic ring is singly, doubly, or triply substituted;

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or



where R^{'''} is a fixed aromatic ring or substituted fused aromatic ring; or



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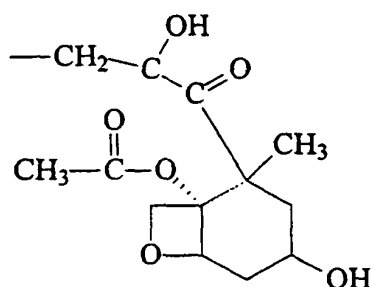
wherein R^{'''} can be H or a short nonsubstituted or substituted hydrocarbon chain C_n wherein n = 1-3 or cyclopropane;

One or more substitution can be made on the aromatic ring of R₄. Preferably, the substituent(s) on the substituted aromatic ring is an electron withdrawing substituent. Examples include fluoro- and chloro-substitution, but any electron-withdrawing substituent

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compatible with the system may be used which provides a lower energy gap in a $\pi - \pi$ interaction between the composition and aromatic amino acids of proteins;

wherein R_5 is one of the following:



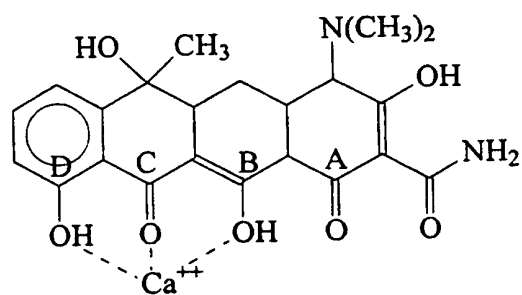
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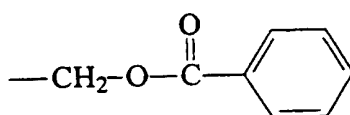
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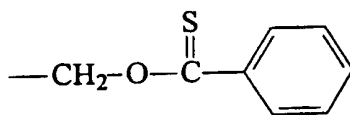
or H; or CH_3 ; or small nonsubstituted or substituted hydrocarbon C_n where $n = 1-5$; or small nonsubstituted or substituted hydrocarbon ring or heterocyclic ring; or citric acid and derivatives thereof; or acetic acid and derivatives thereof; or ascorbic acid and derivatives thereof; or glucouroic acid or derivatives thereof; or lactose, sialic acid, or monosaccharides or disaccharides of glyceraldehyde, erythrose, threose, ribose, arabinose xylose lyxose, allose, altrose, glucose, mannose, gulose, idose, galactose, talose, or their acidic ketose, alditol or inositol forms; or calcium chelating molecule or oxygenated small molecule, i.e., small carboxylic acids; or a dipeptide such as "ASP-ASN" or "GLY-GLN", a cyclic dipeptide such as "PHE-GLN", or small organic molecules that mimic the functional properties of these peptides; or any organic molecule that exhibits calcium-binding properties similar to tetracyclin as given below



or

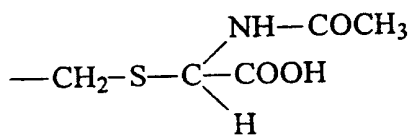


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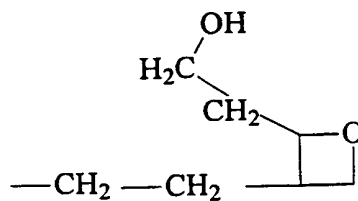


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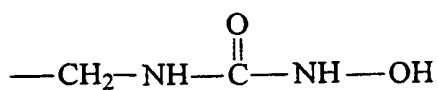


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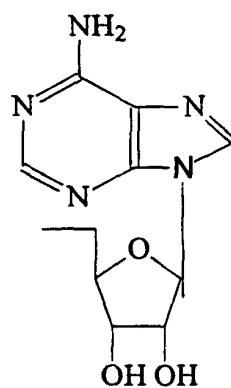


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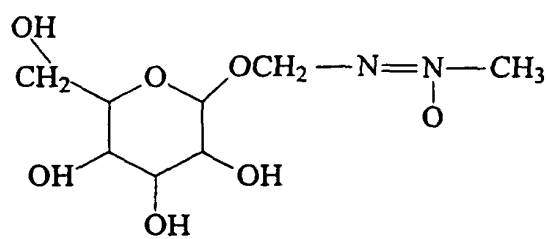
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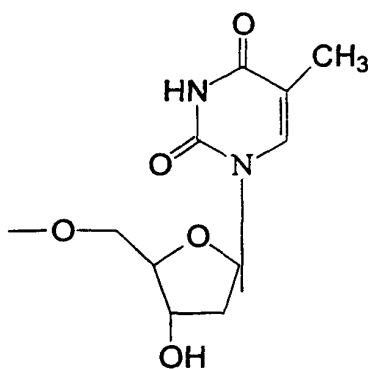


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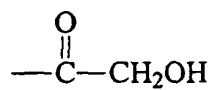
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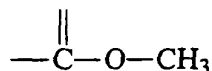
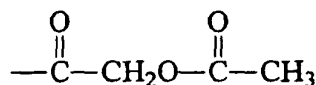
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or in some cases can also be any of the R₄ groups;

wherein R₆ and/or R₆' , which can be the same or different, is one of the following:



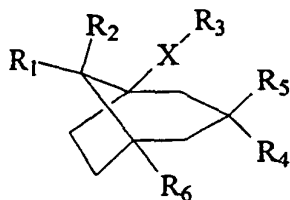


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or H; or CH₃; or OH; or amine or short carbo-aliphatic chain, substituted with two or three of the following: keto, hydroxy, sulfoxy, amide, or an amino acid residue such as serine, asparagine, or threonine; or ethers of the form -CH₂-O-(CH₂)_n-CH₃ where n=1-5 and the right hand hydrocarbon chain may be substituted with up to five -OH or carbonyl groups;

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In another aspect the invention is a paclitaxel compound having the following bicyclo-octane chemical structure



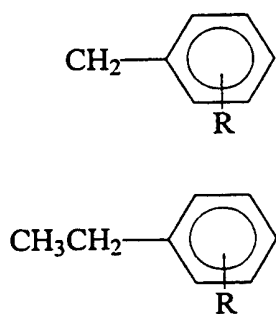
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wherein R₁ or R₂ or both R₁ and R₂ are hydrogen, methyl, acetyl, ethyl, short aliphatic chain (C₁ - C₄), or substituted aliphatic chain (C₁ - C₆) where substitution includes in one or two of the R₁ organic functional groups such as an amide; ketone; hydroxy; phenyl; carboxylic acid; an amino acid, for example, asparagine, glutamine, aspartic acid, glutamic acid, threonine, serine or tyrosine. Preferable chemical structures are obtained with the following:

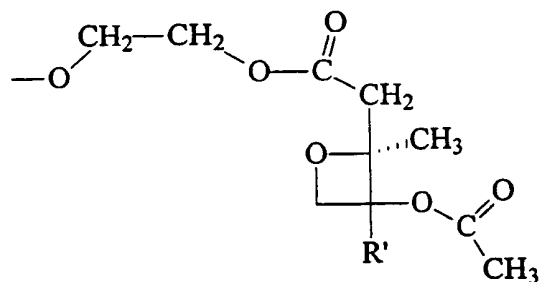
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R₁ = H or CH₃;

R₂ = CH₃; CH₂OCOCH₃; or



wherein R is H or singly, doubly, or triply substituted or fused; or

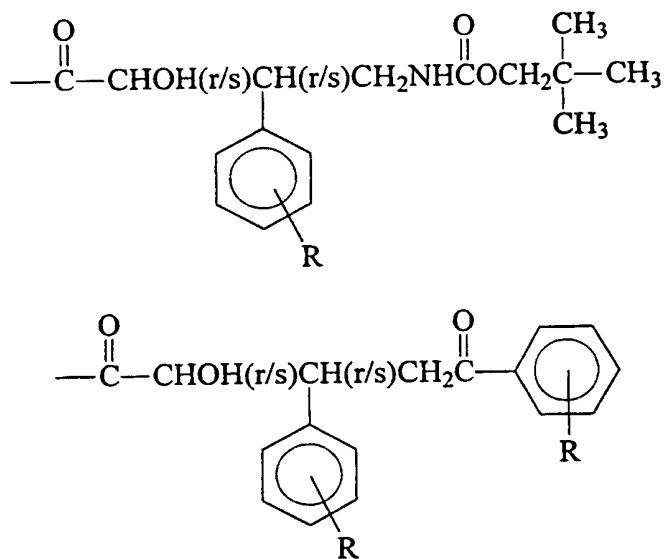


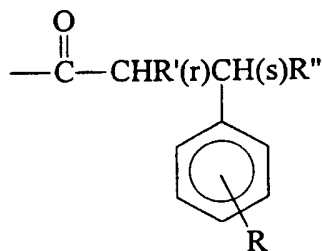
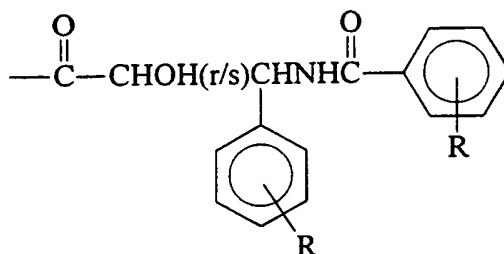
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wherein R' is H or CH_3 ;

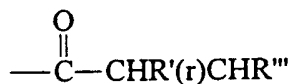
wherein X = O; CH_2 ; NH; S; S- CH_2 ; O- CH_2 or none;

wherein R_3 is one of the following:

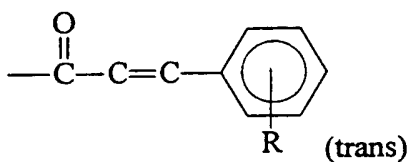




wherein R' = OH when R'' = NHBOC; R' = H when R'' = NHBOC; R' = OH when R'' = H; R' = H when R'' = H
 (These substituents are still active in paclitaxel per
 Guenard, et al. 1993. "Structure-activity relationships of
 taxol and taxotere analogues," *J Natl Cancer Inst*
Monogr 15:79-82.);



wherein R' is as given above and R''' can also be
 substituted aryl (single or double) or fused aromatic ring
 as in tryptophan or imidazol ring, or substituted
 tryptophan; preferably, the aromatic ring can be
 substituted with carboxylic acid derivatives;

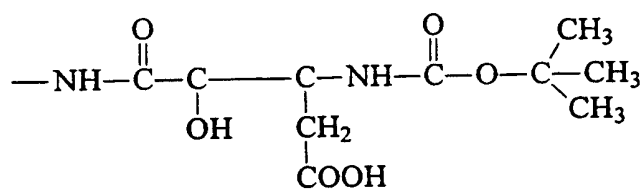
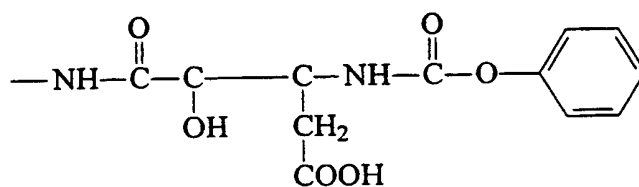


$$\begin{array}{c} \text{O} \qquad \qquad \qquad \text{O} \\ \parallel \qquad \qquad \qquad \parallel \\ -\text{C}-\text{CHOH}(\text{r})\text{CH}(\text{s})\text{NH}-\text{C}=\text{CHCH}_3 \\ \qquad \qquad \qquad | \\ \qquad \qquad \qquad \text{CH}_3 \\ \qquad \qquad \qquad | \\ \qquad \qquad \qquad \text{C}_6\text{H}_4 \\ \qquad \qquad \qquad | \\ \qquad \qquad \qquad \text{R} \end{array}$$

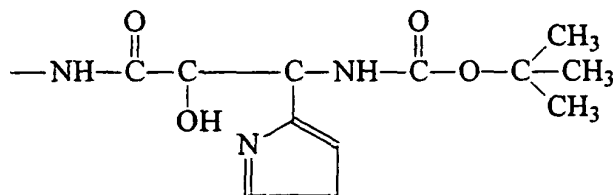
(cephalomannine)

NC(=O)C(O)C1=CN=CC=C1NC(=O)Oc2ccccc2

or

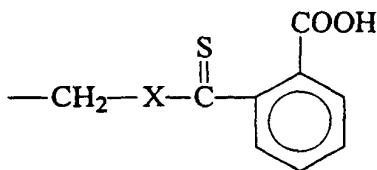

$$\begin{array}{ccccccc} & \text{O} & & & \text{O} & & \text{CH}_3 \\ & \parallel & & & \parallel & & / \\ -\text{NH} & -\text{C} & -\text{C} & - & \text{C} & -\text{NH} & -\text{C} & -\text{O}-\text{C} & \text{CH}_3 \\ & & | & & | & & & & / \\ & & \text{OH} & & \text{CH}_2 & & & & \text{CH}_3 \\ & & & & | & & & & \\ & & & & \text{COOH} & & & & \end{array}$$

18

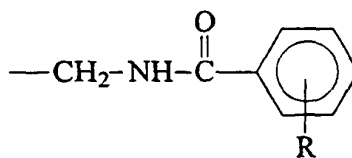
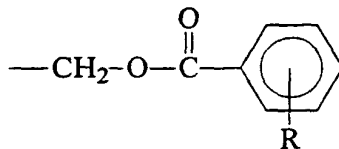


5 For the aryl groups in R₃, R can be H or singly, doubly, or triply substituted OH or preferably with electron withdrawing substituents such as fluoro (F⁻) or trifluoromethyl (CF₃). R₃ can also be any group derived from the 13 position in taxane's skeleton that exhibits activity toward inhibiting the depolymerization of microtubules and/or anticancer activity;

wherein R₄ is one of the following:



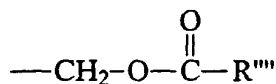
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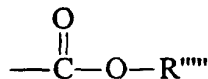
when the aromatic ring is singly, doubly, or triply substituted;

or

15



where R''' is a fixed aromatic ring or substituted fused aromatic ring; or



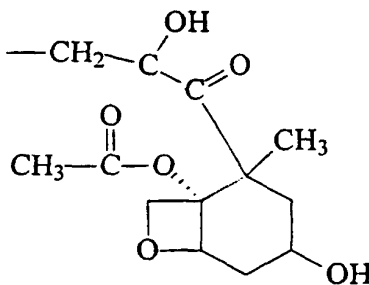
5

wherein R''' can be H or a short nonsubstituted or substituted hydrocarbon chain C_n wherein n = 1-3 or cyclopropane;

10

One or more substitution can be made on the aromatic ring of R₄. Preferably, the substituent(s) on the substituted aromatic ring is an electron withdrawing substituent. Examples include fluoro- and chloro-substitution, but any electron-withdrawing substituent compatible with the system may be used which provides a lower energy gap in a π - π interaction between the composition and aromatic amino acids of proteins;

wherein R₅ is one of the following:



15

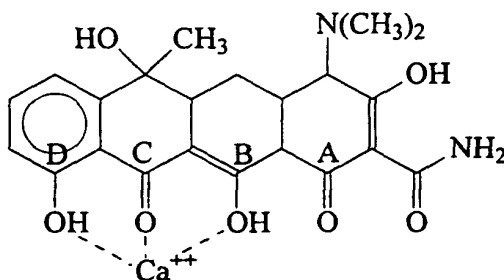
20

or H; or CH₃; or small nonsubstituted or substituted hydrocarbon C_n where n = 1-5; or small nonsubstituted or substituted hydrocarbon ring or heterocyclic ring; or citric acid and derivatives thereof; or acetic acid and derivatives thereof; or ascorbic acid and derivatives thereof; or glucouroic acid or derivatives thereof; or lactose, sialic acid, or monosaccharides or disaccharides

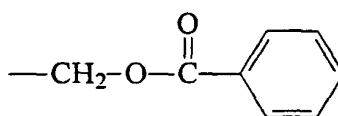
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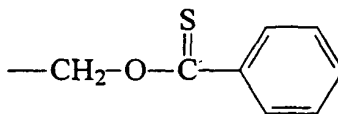
of glyceraldehyde, erythrose, threose, ribose, arabinose
 xylose lyxose, allose, altrose, glucose, mannose, gulose,
 idose, galactose, talose, or their acidic ketose, alditol or
 inositol forms; or calcium chelating molecule or
 oxygenated small molecule, i.e., small carboxylic acids;
 or a dipeptide such as "ASP-ASN" or "GLY-GLN", a
 cyclic dipeptide such as "PHE-GLN", or small organic
 molecules that mimic the functional properties of these
 peptides; or any organic molecule that exhibits calcium-
 binding properties similar to tetracyclin as given below



or

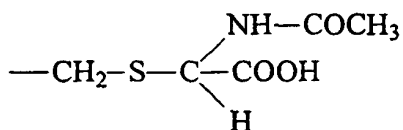


or

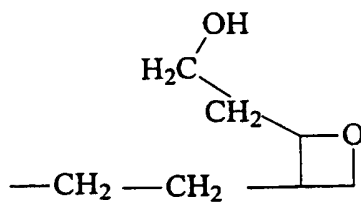


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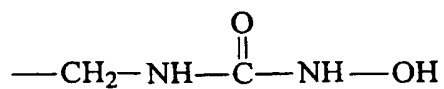
or



or

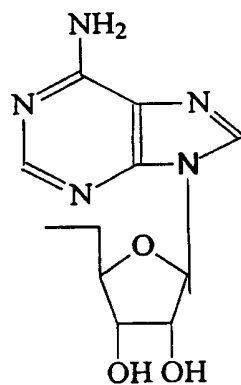


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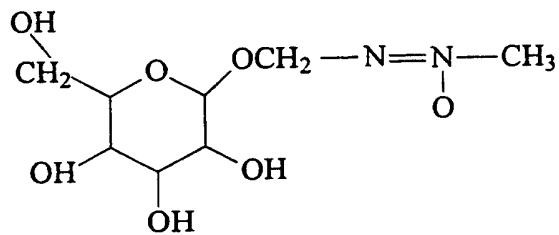


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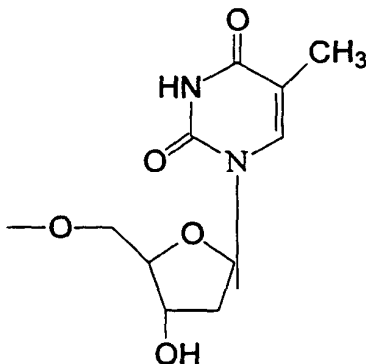
or



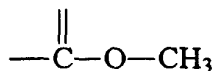
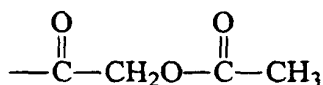
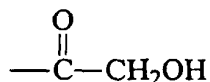
or



or



or in some cases can also be any of the R_4 groups;
 wherein R_6 and/or R_6' , which can be the same or different, is one of the following:



5

10

or H; or CH_3 ; or OH; or amine or short carbo-aliphatic chain, substituted with two or three of the following: keto, hydroxy, sulfoxy, amide, or an amino acid residue such as serine, asparagine, or threonine; or ethers of the form $\text{—CH}_2\text{—O—(CH}_2\text{)}_n\text{—CH}_3$ where $n=1-5$ and the right hand hydrocarbon chain may be substituted with up to five -OH or carbonyl groups;

BRIEF DESCRIPTION OF THE DRAWINGS

15

Fig. 1A-1E depict the five steps of the computational drug design method used. Fig. 1A depicts the structure of paclitaxel in the tubulin binding site with the paclitaxel skeleton and non-active side chains removed and the position of the modified side chain, C3 ester group and oxetane oxygen atom maintained. Fig. 1B depicts the

addition of a selected central skeleton, bicyclo[3.2.1]octane. Fig. 1C depicts the connection of the ester group using a CH₂ group. Fig. 1D depicts the addition of a chain that allows the remaining oxygen to be in the correct location. Fig. 1E depicts the addition of an acetyl group to the new skeleton to mimic the paclitaxel C10 acetyl.

5 Fig. 2 depicts the synthesis scheme resulting in the diene **D** which is the reactant for the Diels-Alder reaction.

 Fig. 3 depicts the Diels-Alder reaction between Substance (**D**) and Substance (**H₁**) followed by the combinatorial addition of chlorinated carbohydrate and subsequent removal of the t-butyl-dimethyl-silyl protective group (TBDMS) to yield
10 the substituted norbornyl Product (**V₁**).

 Fig. 4 depicts the Diels-Alder reaction between Substance (**D**) and Substance (**H₂**) followed by the combinatorial addition of chlorinated carbohydrates and other small functionalized Ryodow carbon chains and rings, and subsequent removal of the t-butyl-dimethyl-silyl protective group (TBDMS) to yield the substituted norbornyl
15 Product (**V₂**).

 Fig. 5 depicts the Diels-Alder reaction between Substance (**D**) and Substance (**H₃**) followed by the combinatorial addition of chlorinated carbohydrate and subsequent removal of the t-butyl-dimethyl-silyl protective group (TBDMS) to yield the substituted norbornyl Product (**V₃**), wherein n=2 or 3.

20 Fig. 6 depicts the preparation of norbornene-1,4-diester (**I**). Two methods of preparation are presented: Route A beginning from 1,4-dicarboxymethyl-ester-cyclohexadiene and Route B beginning from munoic acid.

 Fig. 7 depicts the preparation of the diol diester Substance (**K₆**) starting from norbornene-1,4-diester, Substance (**I**).

25 Fig. 8 depicts the preparation of the substituted norbornyl Product (**V₁**) from the diol diester Substance (**K₆**).

Fig. 9A-9G depicts schemes for synthesizing substituted bicyclo-octanes.

DETAILED DESCRIPTION

A method of synthesizing tubulin polymerization stabilizers has now been found. These stabilizing compounds in solution possess steric conformational
5 properties of natural paclitaxel and are capable of binding to the tubulin protein at the same site where paclitaxel is known to bind and with the same or stronger binding energies as paclitaxel. These compounds stabilize tubulin polymerization in a way that is mechanistically equivalent to activity mechanism of paclitaxel. The compounds made by the present invention are paclitaxel-alternative molecules and are
10 referred to hereinafter as the "EB compounds." These compounds have increased solubility, simpler synthesis, and the possibility for specificity and optimization due to the combinatorial reactions over natural paclitaxel.

Computational molecular modeling studies were used to define the properties of the EB compounds necessary to mimic the shape and binding of paclitaxel in
15 tubulin. These studies were performed using the molecular mechanics force fields CHARMM and MMFF; however, it would be understood by one of skill in the art that other force fields could also be used. Conformational search and docking procedures were used to compute both the conformational energies and binding in the tubulin active site. These techniques are commercially available in software packages that
20 include but are not limited to QUANTA™ (Molecular Simulations, Inc., San Diego CA, U.S.A.), Insight II® and associated modules (Molecular Simulations, Inc., San Diego CA, U.S.A.), Cerius²® and associated modules (Molecular Simulations, Inc., San Diego CA, U.S.A.), Molecular Operating Environment (MOE; Chemical Computing Groups, Inc., Montreal, Quebec, Canada), and CHARMm® (Molecular
25 Simulations, Inc., San Diego CA, U.S.A.).

The EB compounds of the present invention are designed using the following computational modeling technique.

Step 1: The structure of a compound known to have anti-tumor activity is obtained in its active conformation, e.g. in the tubulin binding site;

Step 2: The central skeleton is eliminated from the structure, along with any side chains known not to be crucial to its activity;

5 *Step 3:* A new central skeleton is built that will hold the side chains not removed in Step 2 in the same orientation. Additional functional groups can be optionally added to mimic the sterics of the active compound (i.e., its overall shape);

10 *Step 4:* Conformation search techniques are used to find an energetically accessible conformer of the compound with a conformation similar to that of the active conformer;

Step 5: A docking calculation is used to determine the binding energy for the new compound, in the conformation from Step 4, in the tubulin binding site;

Step 6: Further changes are optionally made to the structure in order to improve the binding characteristics.

15

 The EB compounds of the present invention are all characterized by the following structural and physicochemical features:

20 (1) *Central skeleton:* a central skeleton structure composed of single or multiple ring groups which include norbornyls, borane, noradamantane, adamantane, biotin, cyclopropane, cyclobutane, cyclopentane, cyclohexane, cyclohexene, cycloheptane, and bicyclo-octane. The skeleton holds multiple functional groups in a fairly rigid alignment equivalent to the taxane skeleton. Structurally, the skeleton replaces the six membered hexene ring in paclitaxel. For the biotin skeleton, the two fused five membered rings act to structurally replace the paclitaxel hexene ring. The
25 central skeleton structure has various side chains labeled as R groups.

 (2) *Side Chain 1:* a side chain connected to the central skeleton with a carbonyl group at a distance of about 1.5 to 5.5 Angstroms from the skeleton. This is the taxane iso-serine group or R₃;

30 (3) *Side Chain 2:* a side chain that places an sp³ oxygen atom at a distance of about 4.5 to 7.5 Angstroms from the skeleton. One conformation of this side chain should allow this oxygen atom to be oriented in space similar to that of the oxygen in

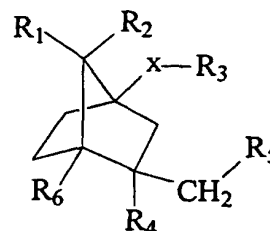
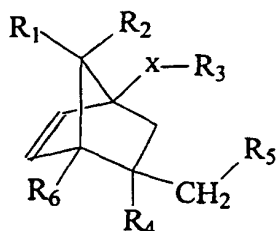
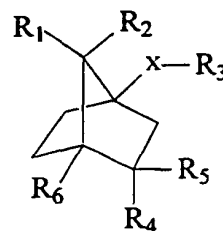
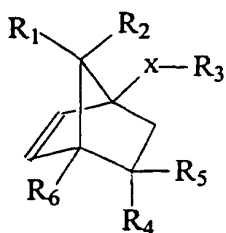
the oxetane ring of paclitaxel. Specifically, the oxygen should be about 9 to 11 Angstroms from the carbonyl oxygen mentioned in Side Chain 1 above in one of the energetically accessible conformers of the molecule. This is the R_5 group and in some cases the R_2 group;

- 5 (4) *Side Chain 3*: a group that mimics the steric and binding properties of the C2 ester in paclitaxel. This is R_7 or R_4 or some of the larger R_2 groups. The groups should be able to adopt an energetically accessible conformation that places an aromatic ring in a location that is simultaneously about 4 to 6 Angstroms from the hexene substitute, about 8 to 10 Angstroms from the carbonyl oxygen specified in part
- 10 (2) above, and about 4 to 6 Angstroms from the oxygen specified in Side Chain 2 above; and

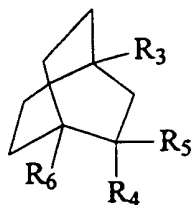
(5) additional groups that allow the overall size and shape of the molecule to be similar to paclitaxel. These are the groups R_1 , R_2 , R_6 and in some cases R_4 .

Representative chemical structures for the EB compounds are given below:

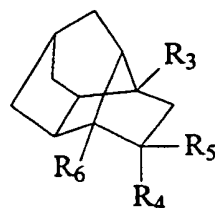
15 norbornyls



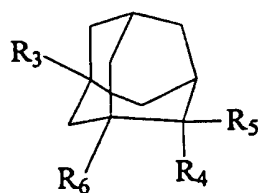
borane



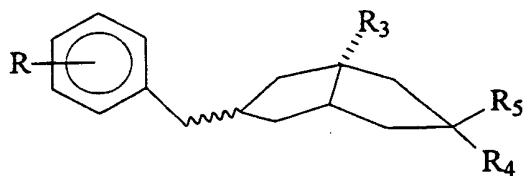
noradamantane



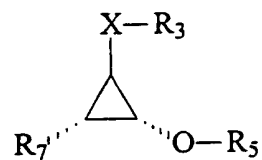
5 adamantane



biotin

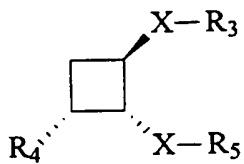


cyclopropane

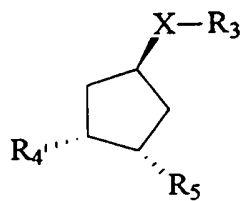


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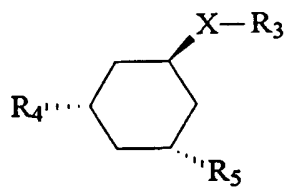
cyclobutane



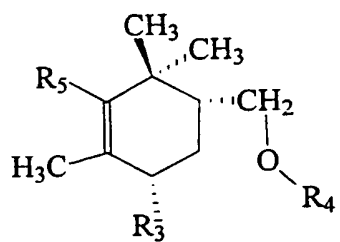
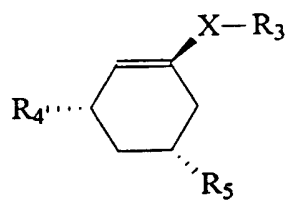
cyclopentane



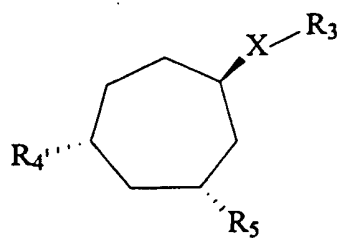
5 cyclohexane



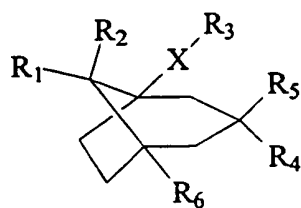
cyclohexene



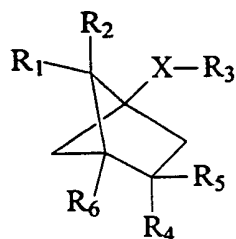
cycloheptane



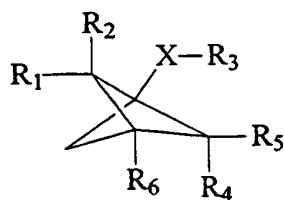
bicyclo[3,2,1]octane



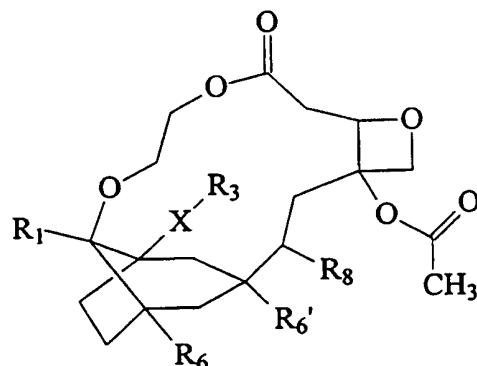
5 bicyclo[2,1,1]hexane



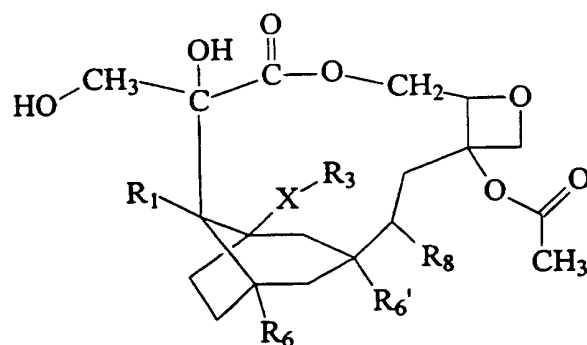
bicyclo[1,1,1]pentane



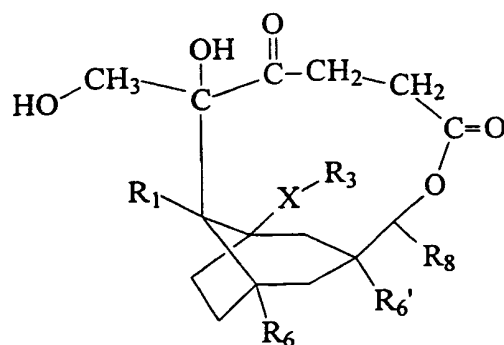
heterocyclic compound I



heterocyclic compound II



5 and heterocyclic compound III

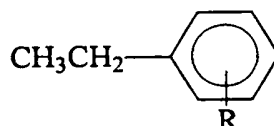
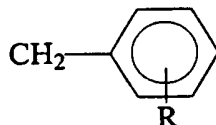


wherein R₁ or R₂ or both R₁ and R₂ are hydrogen, methyl, acetyl, ethyl, short aliphatic chain (C₁ - C₄), or substituted aliphatic chain (C₁ - C₆) where substitution includes in one or two of the R₁ organic functional groups such as an amide; ketone; hydroxy; phenyl; carboxylic acid; an amino acid, for example,

asparagine, glutamine, aspartic acid, glutamic acid, threonine, serine or tyrosine. Preferable chemical structures are obtained with the following:

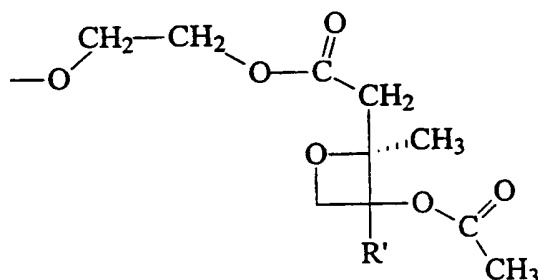
$R_1 = \text{H or CH}_3$;

$R_2 = \text{CH}_3$; $\text{CH}_2\text{OCOCH}_3$; or



5

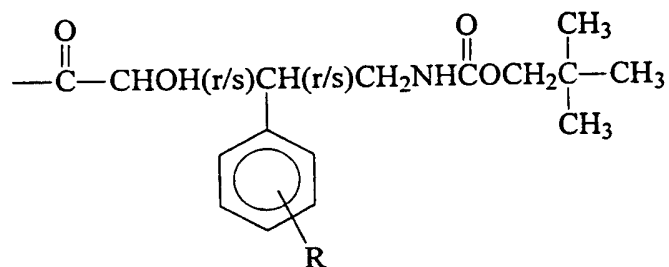
wherein R is H or singly, doubly, or triply substituted or fused; or

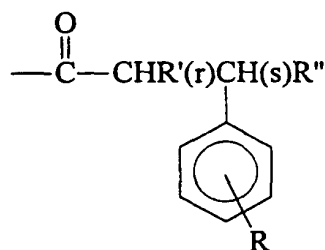
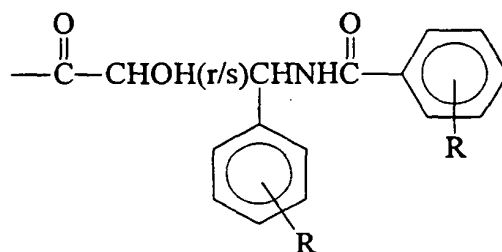
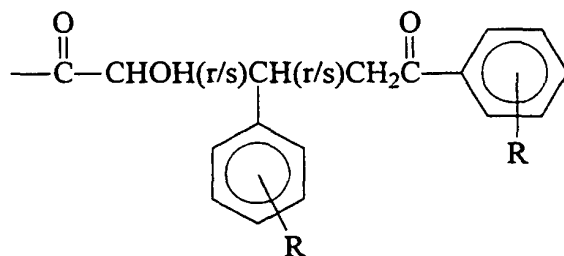


wherein R' is H or CH_3 ;

10 wherein X = O; CH_2 ; NH; S; S- CH_2 ; O- CH_2 or none;

wherein R_3 is one of the following:

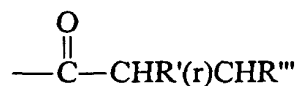




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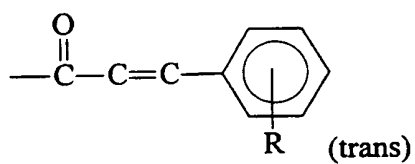
wherein $R' = \text{OH}$ when $R'' = \text{NHBOC}$; $R' = \text{H}$ when $R'' = \text{NHBOC}$; $R' = \text{OH}$ when $R'' = \text{H}$; $R' = \text{H}$ when $R'' = \text{H}$
 (These substituents are still active in paclitaxel per
 Guenard, et al. 1993. "Structure-activity relationships of
 taxol and taxotere analogues," *J Natl Cancer Inst*
Monogr 15:79-82.);

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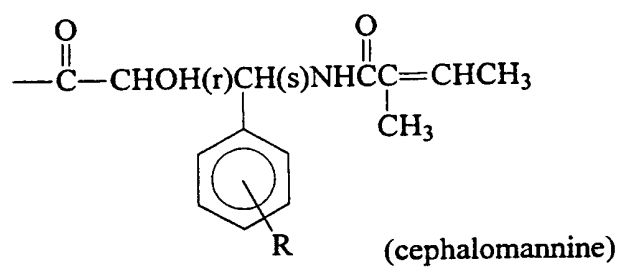


wherein R' is as given above and R''' can also be
 substituted aryl (single or double) or fused aromatic ring
 as in tryptophan or imidazol ring, or substituted

tryptophan; preferably, the aromatic ring can be substituted with carboxylic acid derivatives;

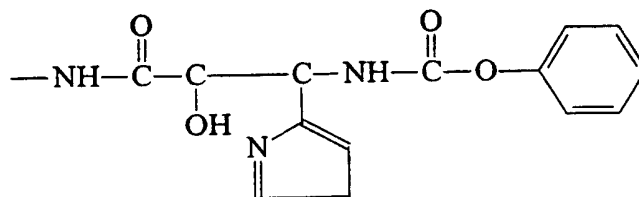


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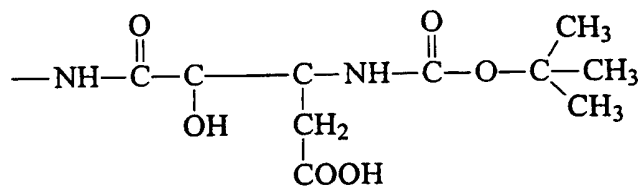
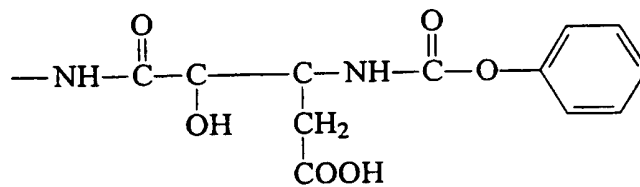


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or

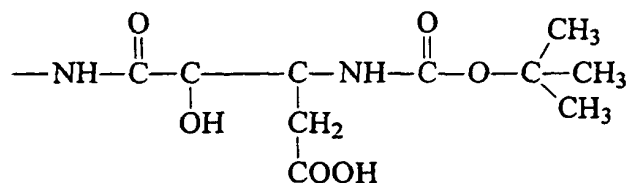


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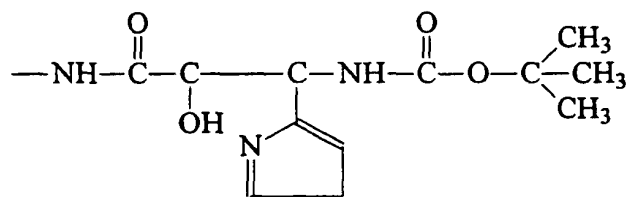


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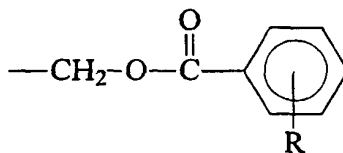
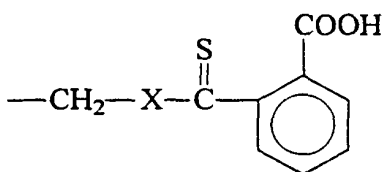
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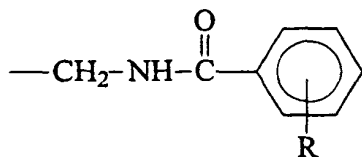


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For the aryl groups in R₃, R can be H or singly, doubly, or triply substituted OH or preferably with electron withdrawing substituents such as fluoro (F⁻) or trifluoromethyl (CF₃⁻). R₃ can also be any group derived from the 13 position in taxane's skeleton that exhibits activity toward inhibiting the depolymerization of microtubules and/or anticancer activity;

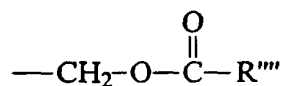
10 wherein R₄ is one of the following:





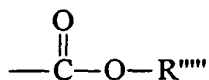
when the aromatic ring is singly, doubly, or triply substituted;

or



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where R'''' is a fixed aromatic ring or substituted fused aromatic ring; or



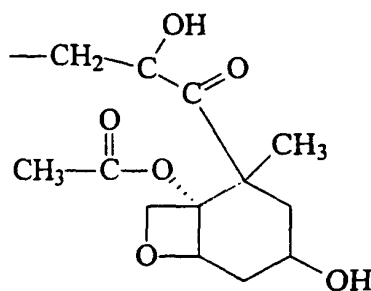
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wherein R'''' can be H or a short nonsubstituted or substituted hydrocarbon chain C_n wherein n = 1-3 or cyclopropane;

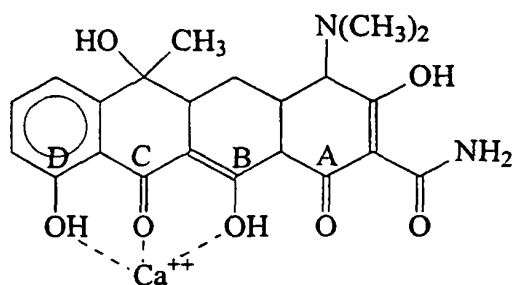
15

One or more substitution can be made on the aromatic ring of R₄. Preferably, the substituent(s) on the substituted aromatic ring is an electron withdrawing substituent. Examples include fluoro- and chloro-substitution, but any electron-withdrawing substituent compatible with the system may be used which provides a lower energy gap in a π - π interaction between the composition and aromatic amino acids of proteins;

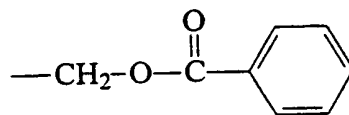
wherein R₅ is one of the following:



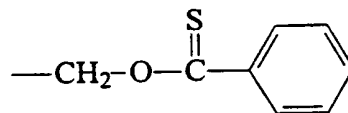
or H; or CH₃; or small nonsubstituted or substituted
 hydrocarbon C_n where n = 1-5; or small nonsubstituted
 or substituted hydrocarbon ring or heterocyclic ring; or
 citric acid and derivatives thereof; or acetic acid and
 derivatives thereof; or ascorbic acid and derivatives
 thereof; or glucouroic acid or derivatives thereof; or
 lactose, sialic acid, or monosaccharides or disaccharides
 of glyceraldehyde, erythrose, threose, ribose, arabinose
 xylose lyxose, allose, altrose, glucose, mannose, gulose,
 idose, galactose, talose, or their acidic ketose, alditol or
 inositol forms; or calcium chelating molecule or
 oxygenated small molecule, i.e., small carboxylic acids;
 or a dipeptide such as "ASP-ASN" or "GLY-GLN", a
 cyclic dipeptide such as "PHE-GLN", or small organic
 molecules that mimic the functional properties of these
 peptides; or any organic molecule that exhibits calcium-
 binding properties similar to tetracyclin as given below



or

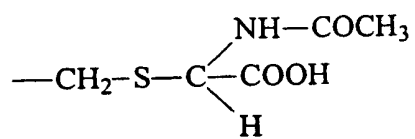


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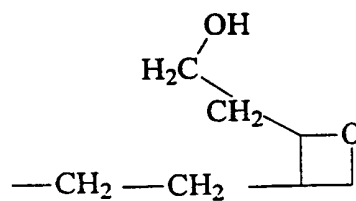


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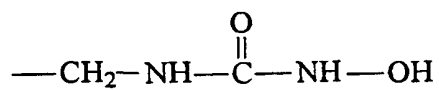
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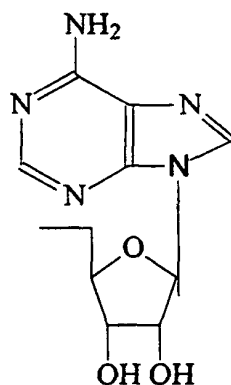


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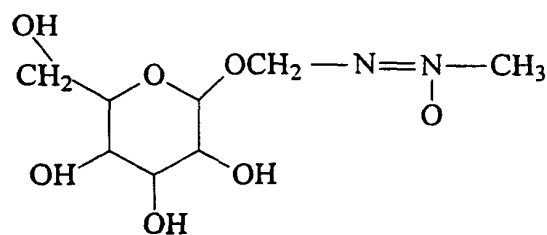


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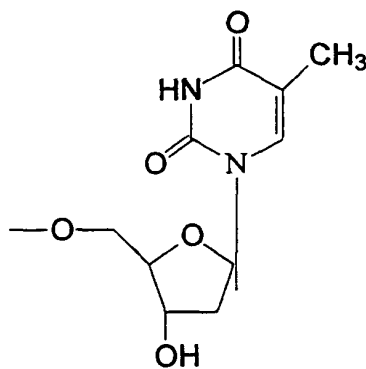
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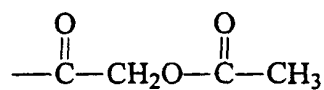
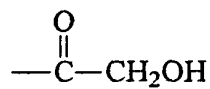
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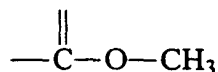


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or in some cases can also be any of the R_4 groups;

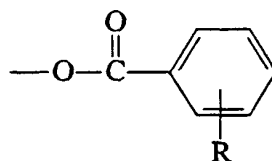
wherein R_6 and/or R_6' , which can be the same or different, is one of the following:





5 or H; or CH₃; or OH; or amine or short carbo-aliphatic chain, substituted with two or three of the following: keto, hydroxy, sulfoxy, amide, or an amino acid residue such as serine, asparagine, or threonine; or ethers of the form -CH₂-O-(CH₂)_n-CH₃ where n=1-5 and the right hand hydrocarbon chain may be substituted with up to five -OH or carbonyl groups;

wherein R₇ is O-R₄, or NN, or NN-(C=O)-phenyl, or NN-(C=S)-phenyl; and
10 wherein R₈ is the following:



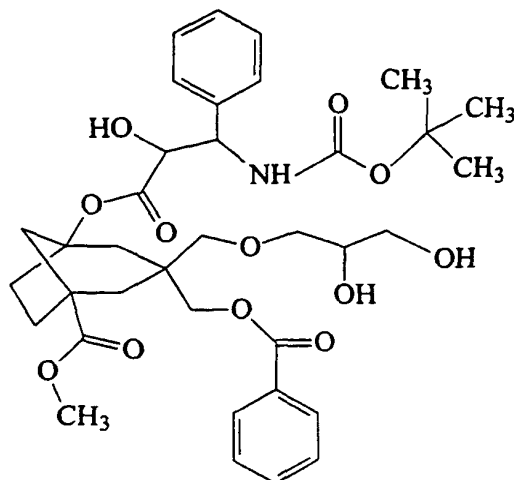
The EB compounds of the present invention can be synthesized using conventional techniques using available starting materials. Representative synthesis schemes are presented in Fig. 2-10E and some of the examples given below. The
15 synthesis methods provided result in racemic mixtures; however, it is within the skill of one in the art to prepare and separate the diastereomers to isolate the preferred chiral compound. All such forms of these compounds are expressly included in the present invention.

20 **Example 1: Designing A Representative EB Compound Using Computational Technique**

The bicyclo[3.2.1] octane compound having the chemical structure given below was designed using the following procedure:

Step 1: The structure of docetaxel in the tubulin binding site was obtained. The docetaxel skeleton and non-active side chains were removed, while holding the

modified side chain, C3 ester group and oxetane oxygen atom in place. This results in the structure illustrated in Fig. 1A.



Step 2: A skeleton was selected which will hold these side chains. Selecting the bicyclo[3.2.1] octane skeleton results in the structure illustrated in Fig. 1B;

Step 3: The ester group was connected to the skeleton. Using a CH₂ group to connect them results in the structure illustrated in Fig. 1C;

Step 4: A chain was constructed that will allow the remaining oxygen atom to be in the correct location. This results in the structure illustrated in Fig. 1D;

Step 5: Functional groups were added so that the new skeleton will take up about the same amount of space as the bulkier docetaxel skeleton. In this case, an acetyl group was added to match the location of the docetaxel C10 acetyl, resulting the structure illustrated in Fig. 1E;

Step 6: Existing conformational search techniques were used to compute a list of conformers of the molecule and their associated conformational energies. If an energetically accessible conformer that is similar in shape to docetaxel in its binding conformation was found, that conformer was used in *Step 7*. Otherwise the process was repeated with different skeleton and R groups;

Step 7: The energetically accessible conformers were used in a docking calculation to determine the binding energy of the compound in the tubulin active site as shown in Example 2; and

Step 8: Compound showing an acceptable binding energy in *Step 7* were then synthesized and assayed.

Example 2: Computation of Binding Energies

5 The binding energies were obtained computationally for the EB compound given in Example 1 by the following procedure:

Step 1: The molecular structure was obtained according to Example 1.

Step 2: Computational conformation searches on the molecule were performed using MOE software. The MMFF94 force field was used to compute energies. Both the RIPS and Hybrid Monte Carlo conformation search algorithms
10 were used. This yielded a number of conformers of the molecule along with their relative conformational energies. Some of the lowest energies obtained were:

$$E_1 = 235.0108 \text{ kcal/mol}$$

$$E_2 = 237.0030 \text{ kcal/mol}$$

$$E_3 = 237.8409 \text{ kcal/mol}$$

15 $E_4 = 239.0317 \text{ kcal/mol}$

$$E_5 = 242.6232 \text{ kcal/mol}$$

$$E_6 = 243.2933 \text{ kcal/mol}$$

$$E_7 = 243.6304 \text{ kcal/mol}$$

$$E_8 = 243.7124 \text{ kcal/mol}$$

20 *Step 3:* The conformation of these conformers were examined by overlaying the computed conformer with the structure of a compound known to have a drug activity in its active conformation. For this study, the paclitaxel derivative docetaxel (TAXOTERE®, Rhone-Poulenc Rorer) in its active conformation was obtained from the protein data bank. In this study, the lowest energy compound had a conformation
25 very similar to the active conformation of docetaxel. This conformer was used in the subsequent steps.

Step 4: A model of the binding site was obtained. The structure of tubulin with docetaxel bound in the active site was obtained from the protein data bank. The structure of the tubulin/docetaxel complex was minimized using the MMFF force

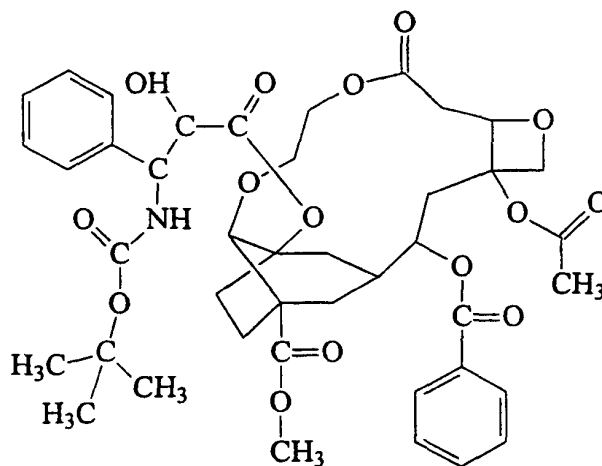
field. The docetaxel was then removed from the structure and the resulting geometry for tubulin was used for the docking calculations.

Step 5: Docking calculations were run using the docking algorithm in the MOE software and the MMFF94 force field. The binding energies that are reported are the sum of the intermolecular electrostatic term and the intermolecular nonbond (or van der Waals) term in the MMFF force field. The binding energy for our compound was -138.7597 kcal/mol, as compared to the binding energy for docetaxel of -134.0277 kcal/mol when compared by the same method.

Step 6: Finding a new compound with an energetically accessible conformation that has a binding energy similar to that of a known active compound is considered a positive result for the computational prediction of new compounds. Compounds showing preferred binding energies were then synthesized.

Example 3: Designing A Representative EB Compound Using Computational Technique and Computation of Binding Energies

The following compound was designed as given in Example 1 and the binding energies were determined as given in Example 2.



The binding energy for this compound was determined to be -165.7084 kcal/mol.

Example 4: Synthesis Of A Substituted Norbornyl

Synthesis of Diene Reactant (Substance D) for Diels - Alder Reaction

Now referring to Fig. 2, which sets forth the synthesis scheme resulting in Diene Substance (D), the starting material was a 2-cyclopenten-1-one molecule which
 5 can be protected in its 5 position by reacting with carboxyl-trimethyl-silane in the presence of lithium di-isopropylamide (LDA) and tetrahydrofuran (THF) to yield Substance (A). Substance (A) then was condensed via a Michael condensation with I-t-butyl-dimethyl-silyloxy-acetic acid-methyl ester to yield condensation Substance (B). An additional Michael condensation (O-alkylation) with a series of acid chlorides
 10 substituted with R₃ would yield the diene Substance (C). Selective desilylation (removal of the TMS-trimethyl-silyl groups) followed by decarboxylation to yield Substance (D) which is the diene reactant for the Diels Alder reaction.

Synthesis of Dienophile (Substance H) for Diels Alder Reaction

The dienophile Substance (H) (H₁ in Fig. 3; H₂ in Fig. 4; H₃ in Fig. 5) was
 15 obtained from a Wittig reaction of dihydroxy acetone and phosphonyl-yilide followed by mono esterification with benzoic acid (or any R₄) to yield Substance (H).

Diels-Alder Reaction

Substance (D) and Substance (H) were mixed under conditions known to the art for Diels-Alder reactions and published in Corey, et al. 1969. *J Am Chem Soc*
 20 91:5675. The product of the Diels-Alder Reaction was an addition product, Substance (G) (G₁ in Fig. 3; G₂ in Fig. 4; G₃ in Fig. 5).

Combinatorial Addition of Carbohydrates or Chelating Agents

Chlorinated carbohydrates or calcium chelators may be reacted with Substance (G) (G₁ in Fig. 3; G₂ in Fig. 4; G₃ in Fig. 5). Shown in Fig. 3 is the reaction of a
 25 chlorinated carbohydrate ("sugar-chloride") to yield the Adduct (L) (L₁ in Fig. 3; L₂ in Fig. 4; L₃ in Fig. 5), which was then reacted with potassium fluoride salt to remove the t-butyl-di-methyl-silyl(t-BuDMSi) protective group to yield the substituted norbornyl Product (V) (V₁ in Fig. 3; V₂ in Fig. 4; V₃ in Fig. 5).

Example 5: Synthesis Of Substituted Norbornyl

Preparation of Norbornene-1,4-diester

Now referring to Fig. 6, two alternatives are shown for the synthesis of norbornene-1,4-diester Substance (I): Route A and Route B. In Route A, 1,4,-dicarboxymethyl-ester-cyclohexadiene, Substance (E), is reacted with diazomethane, according to the procedure of Guha et al, *Chem. Abstr.* 34:2822 (1940) to yield Substance (I). In Route B, munoic acid, Substance (J), was reacted with diazomethane and heated to undergo a rearrangement as described in Guha et al., *Berichte* 70:2109 (1937) to yield cyclopentene dicarboxymethylester, Substance (F). Substance (F) was then reacted with lithium dimethyl amide (LDA) according to Della et al, *Austral. J. Chem* 38:1705 (1985) and 1-bromo-2-chloro-ethane to yield Substance (I).

Preparation of Diol ester

As shown in Fig. 7, Substance (I) was reacted with catecholborane, as described in Brands et al., *Tetrahedron Lett.* 33:5887 (1992), followed by Jones oxidation to yield Substance (K₁). Substance (K₁) was reacted with lithium and pyridine according to Meinwald et al, *J. Org. Chem.* 35:1891 (1970); see also McMurry, *Org. Reactions* 24:188. Substance (K₁) underwent selective nucleophilic demethylation to yield the monoester Substance (K₂). Substance (K₂) was reacted with phosphonyl-ylide (Wittig reaction) according to *Organophosphorus Reagents in Organic Synthesis*. 1979. J.I.G. Coutogan (ed), Academic Press, London, pp. 17-153 and Maryanoff, et al. 1989. *Chem Rev* 89:863 to yield Substance (K₃). Substance (K₃) was reacted with the reagent (COCl)₂ followed by tri-trimethylsilyloxy ethylene, according to Wissner, *J.Org.Chem.* 44:4617(1979) and Kende et al., *Tetrahedron Lett.* 23:1751 (1982), to yield the Substance (K₄). The hydroxy group of Substance (K₄) was then protected with a t-butyl-dimethylsilylchloride (TBDMSCl) to yield Substance (K₅). The carboxyethylester group in position 1 of the norbornyl skeleton in Substance (K₅) then underwent two reactions: formation of the acid chloride carboxyl inversion and O-acylation with the appropriate R₃ group, followed by oxidation of the methylene at the norbornyl 5th position (exo attack) with osmium

tetraoxide (OsO_4) and esterification with benzoyl chloride (or any other R_4 acid chloride group) to yield Substance (K_6).

Combinatorial addition of carbohydrate or chelating agent

Referring to Fig. 8, Substance (K_6) was then reacted with any carbohydrate chloride (also known as “sugar chloride” or “chlorinated carbohydrate”) or parallel reagent to yield Substance (L_1) which was then reacted with HF in pyridine to yield Product (V_1).

Example 6: Tubulin Polymerization Assays

Various assays can be used to demonstrate binding of the EB compounds of the present invention to tubulin.

Tubulin Polymerization Assay.

Tubulin polymerization activity is assayed using the Microtubule/Tubulin Biochem Kit purchased from Cytoskeleton. The assay mixture contains the following:

	10 μl	GTP (100mM)
15	21 μl	DMSO
	200 μl	Tubulin (5 mg/ml)
	130 μl	Test compound (1.5 mM)
	670 μl	PEM (80mM Piperazine-N,N'-bis[2-ethanesulfonic acid] sequisodiumsalt; 0.5 mM Magnesium chloride; 1mM Ethylene glycol-bis(b-amio-ethyl ether) N,N, N', N'-tetra-acetic acid, pH6.9)
20	<hr/>	
	1000 μl	Total Volume

The assay mixture is immediately placed in a Beckman DU640 UV/VIS spectrophotometer at 24°C. Tubulin polymerization is monitored by measuring the absorbance at 340 nm every 60 seconds for one hour. The absorbance plots are then

compared to a paclitaxel standard in order to compare relative tubulin polymerization activities.

Tubulin Polymerization at 37 °C/Incubation with Test Compound.

The absorbance at 340 nm of the mixture listed in the table above is initially
 5 read at 24 °C. The mixture is then transferred to a 37 °C water bath and incubated for
 20 minutes. The absorbance at 340 nm is read then read using a Beckman DU640
 spectrophotometer. Further readings are collected every 20 minutes up to a total of 80
 minutes. The mixture remain at 37 °C between readings. The absorbance trend is
 compared to a standard containing 130 µl of 30% DMSO in place of the test
 10 compound.

Tubulin Polymerization in the Presence of Paclitaxel

In order to determine whether or not the test compounds compete for the
 paclitaxel binding site on the tubulin protein without enhancing polymerization, the
 above mixture containing the test compound is incubated at 37 °C for 20 minutes. At
 15 this time, the mixture is transferred to a Beckman DU640 spectrophotometer and 10
 µM paclitaxel added. The absorbance at 340 nm is measured every 60 seconds for one
 hour and compared to a standard containing 130 µl of 30% DMSO in place of the test
 compound along with 10 µM paclitaxel.

Colorimetric assay for cell viability and proliferation of BT-20 breast cancer cells

20 The following colorimetric assay is used to detect and measure cell viability ,
 activation and proliferation of BT-20 breast cancer cells after incubation with and
 without the paclitaxel compounds of the present invention. Sodium 3'-
 [1[(phenylamino)-carbonyl]-bis(4-methoxy-6-nitro) benzene-sulfonic acid hydrate
 was used in a colorimetric assay for cell viability and proliferation by BT-20 breast
 25 cancer cells. Cleavage of XTT by dehydrogenase enzymes of metabolically active
 cells yields a highly colored formazan product which is water soluble. Bio-reduction
 of XTT can be potentiated by the addition of electron coupling agents such as
 phenazine methosulfate (PMS) or menadione (MEN). Optimal concentrations of PMS

or MEN were determined. Assays were performed essentially as in Roehm, N.W.;
Rodgers, G.H.; Hartfield, S.M.; Glasebrook, A.L.; Journal of immunological Methods,
142 (1991) 257-265. Briefly, solutions of 1 mg/mL XTT in 60°C DMEM media and
25 mM PMS in deionized water are prepared (the XTT solution must be prepared
5 fresh each day, although PMS is stable at 2-8°C in the dark for at least three months).
Then thiol - free DMEM is warmed to 60°C and subsequently 15 µL of the 25 mM
stock solution is mixed with 3 mL of XTT/media giving a PMS concentration of 125
µM. Immediately 25 µL of XTT/PMS solution is added to 100 µL of culture in each
well giving final concentrations of 0.2 mg/mL XTT and 25 µM PMS. Cells were
10 incubated at 37°C for 4-8 hours before reading the absorbance at 470 nm.

Tubulin depolymerization assay

Screening compounds for microtubule destabilization activity are
accomplished with the CytoDYNAMIX Screen™ 6 (Cytoskeleton, Inc.). Briefly,
tubulin are polymerized to steady state by the addition of one volume G-PEM (80 mM
15 piperazine-N,N'-bis[2-ethanesulfonic acid] sequisodium salt, 0.5 mM magnesium
chloride, 1 mM ethylene glycol-bis(b-amino-ethyl ether) N,N,N',N'-tetraacetic acid pH
= 6.9, and 10 mM GTP) plus 10% glycerol and incubating at 37°C for 30 minutes.
Test compounds are then prepared in 120 µL G-PEM in a 96-well plate at 37°C. The
test compound and polymerized tubulin are mixed in a 3:1 ratio. The absorbance at
20 340 nm is measured over time and compared to the paclitexal standard.

The EB compounds of the present invention can be applied to therapeutic
treatments of diseases such as cancer of various type, polycystic kidney disease, and
inflammation and related uses. Generally, any disease which involves cell division
can be addressed by these novel molecules.

25 It should be understood that the compounds of this invention may be modified
by appropriate functionalities to enhance selective biological properties. Such
modifications are known in the art and include those which increase biological
penetration into a given biological system (e.g., blood, lymphatic system, central
nervous system), increase oral availability, increase solubility to allow administration

by injection, alter metabolism and alter rate of excretion. In addition, the compounds may be altered to pro-drug form such that the desired compound is created in the body of the patient as the result of the action of metabolic or other biochemical processes on the pro-drug.

5 The compounds of this invention may be employed in a conventional manner for the treatment of diseases. Such methods of treatment, their dosage levels and requirements would be understood by one of ordinary skill in the art from available methods and techniques. For example, a compound of this invention may be combined with a pharmaceutically acceptable adjuvant for administration to a patient
10 suffering from cancer in a pharmaceutically acceptable manner and in an amount effective to lessen the severity of that disease.

 The EB compounds may be employed in pharmaceutical compositions either alone or together with other compounds of this invention. The compounds of this invention may also be co-administered either concomitantly or sequentially with
15 other therapeutic drugs to increase the effect of therapy. The pharmaceutical compositions can be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, vaginally or via an implanted reservoir. The pharmaceutical compositions are preferably administered by intravenous infusion in water, sodium chloride or any other suitable intravenous infusion solution.

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